THE EFFECTS OF SULFONAMIDE DRUGS AS INFLUENCED BY THE RETICULO-ENDOTHELIAL SYSTEM

BY

FRANK THOMAS MAHER

B.S., University of Illinois, 1937
M.S., University of Illinois, 1938

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1941

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The purpose of this investigation has been a fundamental study of the importance of reticulo-endothelial (R.E.) function to the manifestation of sulfonamide chemotherapy in experimental infections in rabbits.

In order to conduct this investigation on a rational basis, a number of preliminary experiments, relating to the physiology of the R.E.S., were found to be necessary.

Evidence is presented to justify the chemical procedures employed in the determinations of sulfanilamide and sulfathiazole. The suitability of rabbits to this type of investigation is discussed. Bacterial cultures used for the experimental infections are described, and their virulence for rabbits is presented.

Minor Thesis Topics

I. *The Conjugation Phenomenon*

Sulfonamide drugs are determined by a modification of Marshall's method, as previously described by Maher and Camp. Methods involving the use of the photelometer and the comparison colorimeter are described.

Studies in the tissues and body fluids of rabbits have indicated that the rabbit normally conjugates a high percentage of ingested sulfanilamide or sulfathiazole. The general distribution of sulfanilamide throughout the tissues and fluids of rabbits is shown by analyses of samples of these materials following administration of the drug.

Studies in human subjects have shown that, following the ingestion of single doses of sulfanilamide, the conjugated form of the drug does not appear in the urine for periods of time up to two hours after the appearance of the free drug. This lag phase in conjugation is related in discussion to the similar lag phase noted by other workers in the bacteriostatic and chemotherapeutic activity of sulfanilamide and its derivatives.

II. *Procedures Designed to Blockade the R.E.S.*

Preliminary studies were conducted into the use of thorium dioxide, titanium dioxide, silicon dioxide, india ink, egg albumin, finely divided carbon, micron bentonite and ferric oxide in producing R.E. blockade.

From these studies, Thorotrast (colloidal thorium dioxide, Heyden Chemical Company) was selected as the material best suited to the investigation. The low toxicity of this material on intravenous injection, in doses up to 8 cc. per kilogram of body weight of rabbit was demonstrated. Three rabbits now on hand in good condition received a total of approximately 20 cc. of Thorotrast by intravenous injection one year ago.

X-ray studies have shown that thorium dioxide is rapidly immobilized in the spleen and liver of the rabbit, following the intravenous injection of Thorotrast. For a period of four days following injection, no significant changes can be demonstrated in the storage of thorium dioxide. At nine months after the intravenous injection of Thorotrast, a secondary storage of thorium dioxide is demonstrable in the marrow of the ribs, sternum, and vertebral column.

Granules of thorium dioxide are found thickly distributed in sections of livers, lungs, kidneys, and spleens of rabbits, following the intravenous injection of Thorotrast. Such granules are particularly demonstrable by the use of the dark-field microscope.

Following the intravenous injection of Thorotrast, the ability of rabbits so treated to conjugate sulfanilamide and sulfathiazole is significantly depressed or abolished. The most striking effect upon conjugation follows the intravenous injection of three doses of Thorotrast, totaling 6 cc. per kilogram, over a period of 24 hours, followed by the administration of sulfanilamide within a few hours.

The depression in the ability of these rabbits to conjugate sulfanilamide is temporary, with a duration of approximately two days. Following this period of time, the ability of these rabbits to conjugate sulfanilamide is again demonstrable.

These results indicate that the conjugation of sulfanilamide is a chemical function of the cells of the R.E.S. The preliminary and temporary depression of this function by experimental blockade is followed by a proliferation and regeneration of the system, resulting in a resumption of the conjugation phenomenon.

III. *Preliminary Experimental Infections*

Fourteen of the fifteen strains of beta hemolytic streptococcus studied in this investigation were shown to possess a low and irregular virulence for rabbits upon intravenous injection.

Three strains of *Staphylococcus aureus* studied were uniformly virulent for rabbits. Of the strain used, the intravenous injection of 0.6 cc. of a 24-hour culture produced an infection terminating fatally in 24 to 30 hours in a majority of the rabbits so treated.

Sulfathiazole chemotherapy, particularly by the subcutaneous injection of suspensions of the drug in olive oil, afforded a significant degree of protection to normal rabbits against an otherwise fatal staphylococcic bacteremia.

Sulfanilamide was not effective against staphylococcic infection under similar conditions.

IV. *Effect of Thorotrast upon Bacteria*

Intravenous injections of Thorotrast, followed in periods up to 24 hours by the intravenous injection of streptococcic cultures, resulted in an attenuation and dissociation of the streptococci administered. Blockaded animals were more resistant to streptococcic infection than were normal rabbits under similar conditions.

Streptococci cultured in broth containing low concentrations of Thorotrast were attenuated and dissociated.

The strains of *Staphylococcus aureus* used in this study were not altered in virulence or morphology by the presence of Thorotrast, *in vitro* or *in vivo*.

On the basis of these findings, staphylococcic infections were employed in this investigation. We have concluded that any investigation into the function of the R.E.S., in which experimental blockade is used, must include data indicating that the material used in producing the blockade is not in itself inimical to the bacterial cultures employed.

V. *Effect of Blocking Procedures upon the Resistance of Rabbits to Experimental Staphylococcic Infection*

Following the intravenous injection of Thorotrast, the ability of such rabbits to resist experimental staphylococcic infection is markedly reduced, as compared to the response of normal rabbits under identical conditions. In the blockaded rabbits, the course of events leading to fatal termination occurs with greater rapidity and uniformity than in the accompanying normal controls.

Optimal results are obtained following the intravenous injection of a single dose of Thorotrast, amounting to 3 cc. per kilogram of body weight of rabbit. Experimental infections should be administered within six hours after the blockade, to avoid the conflicting results associated with proliferation and regeneration of R.E. function.

The depression in the ability of rabbits to resist infection may be demonstrated for approximately two days after the administration of the Thorotrast blockade. Subsequent to this time, a rise in the resistance of such rabbits is noted. At four days after the blockade, such rabbits are more resistant to infection than are normal rabbits under identical conditions.

Evidently a temporary period of R.E. depression produced by the experimental blockade is followed by a period of hyperactive function.

VI. *Studies in Bacteriostasis in vitro*

Using inocula of streptococci and staphylococci far less than those administered to rabbits, and culturing these in broth containing concen-

trations of sulfanilamide and sulfathiazole in excess of those obtained in the blood of rabbits responding favorably to therapy, we have not been able to demonstrate a bacteriostatic activity as a property of these drugs. Growth in the drug-media occurred in every instance at a rate and extent paralleling that observed in normal media under similar circumstances.

Major Thesis Topic

The major thesis problem consisted of studies in the effect of Thorotrast blockade upon the chemotherapeutic efficiency of sulfathiazole in experimental staphylococcic infections in rabbits, as described below.

I. *Infections Administered During Acute Blockade*

Each experiment in this investigation was completed as a unit, complete in itself. In each, four groups of rabbits were included:

(1) Rabbits submitted to Thorotrast blockade and to staphylococcic infection, treated with sulfathiazole.

(2) Rabbits submitted to Thorotrast blockade and to staphylococcic infection, but untreated.

(3) Normal rabbits, receiving staphylococcic infection, with sulfathiazole chemotherapy.

(4) Normal rabbits, receiving staphylococcic infection only.

By thus including test and control animals, both blocked and unblocked, in each experiment, variations in environment, in procedure, in infection, and in therapy were controlled insofar as possible.

Optimal results were obtained by the following procedure:

(1) Experimental blockades were produced by the intravenous injection of single doses of 3 cc. per kilogram of Thorotrast. Such doses were not toxic to normal rabbits, and did not demonstrably enhance the toxicity of sulfathiazole.

(2) Four hours after the injection of Thorotrast, the calculated dose of staphylococcus culture was injected intravenously into each rabbit of the group.

(3) At a stated time after the infection, preferably within one hour, each of the drug-treated normal and blockaded rabbits in the experiment received the sulfathiazole regimen designated for that experiment. Best results were obtained with subcutaneous injections of sulfathiazole suspended in olive oil.

Results may be summarized as follows:

(1) The experimental infections terminated fatally in normal rabbits, in the absence of therapy, in 24 to 30 hours in a significant majority of the rabbits so treated.

(2) Sulfathiazole chemotherapy afforded a significant protection to normal rabbits against staphylococcic infection. Life periods were extended from four to twenty days, with apparent cures in a number of instances.

(3) Untreated, blockaded rabbits succumbed to infections in a shorter time interval, and with greater uniformity, than did normal rabbits receiving similar infecting inocula. The life period of such blockaded rabbits following infection was 10 to 18 hours in most instances, as compared to 24 to 30 hours for the normal controls.

(4) Sulfathiazole chemotherapy under the same conditions as that effective in normal rabbits did not afford a demonstrable degree of protection to rabbits submitted to Thorotrast blockade. Treated blockaded rabbits succumbed to infection in a manner and at time intervals paralleling the response of the untreated, blockaded groups.

II. *Infections Administered after Recovery from Experimental Blockade*

Preceding studies had shown that, at four days after the administration of R.E. blockade by intravenous injections of Thorotrast, rabbits so treated were again capable of conjugating sulfonamide drugs, and of resisting staphylococcic infection in a manner similar to and even exceeding the abilities of normal rabbits. It became of interest, therefore, to determine the efficacy of sulfonamide therapy in rabbits at this time interval after experimental blockade.

Each experiment in this investigation was conducted as a unit complete in itself, with the four groups of rabbits as described under acute blockade. Infections were administered at four days after experimental blockade, instead of at the four-hour interval described under acute blockade.

Results may be summarized as follows:

(1) The behavior and response of the normal treated and untreated rabbits in these experiments duplicated the results described under acute blockade.

(2) Untreated, blockaded rabbits were capable of resisting infection in a manner paralleling the response of normal untreated rabbits. With the infecting inocula used, these untreated, blockaded rabbits succumbed to infection in 24 to 30 hours in most instances. In some rabbits, evidences of a hyperactive defense mechanism were apparent.

(3) Sulfathiazole chemotherapy afforded a significant degree of protection in drug-treated, blockaded rabbits, in which the infections were administered four days after the blockade. These treated blockaded rabbits responded to therapy in a manner paralleling and often exceeding the response of normal, drug-treated rabbits.

III. *Studies in the Relationships between Bacterial Population, Drug Concentrations in Blood, and Response to Therapy in Blockaded and Normal Rabbits at Uniform Time Intervals after Infection with Varying Infecting Inocula*

Each of the experiments under this heading involved ten to twelve rabbits. Four groups of normal rabbits, and two groups of rabbits, each of which received a single intravenous injection of 3 cc. of Thorotrast per kilogram, were included in the study. Infecting inocula were uniform for the members of each individual group, and included intravenous injections of 0.4 cc., 0.6 cc., 0.8 cc., and 1.0 cc. of 24-hour culture in the normal rabbits, and of 0.4 cc. and 0.6 cc. in the blockaded groups. In the blockaded groups, infections were administered at four hours after the experimental blockade.

Each experiment in this portion of the investigation included drug-treated rabbits, and untreated controls.

At 24 hours after infection, or at the death of the animal if occurring prior to this time, the condition of each animal was noted. Blood samples were drawn from the heart, and the concentrations of free and conjugated sulfathiazole present were determined. In similar blood samples, bacterial populations were determined by the serial dilution and pour plate method.

Results in these experiments may be summarized as follows:

(1) Untreated normal rabbits and untreated blockaded rabbits responded to infection as described in previous experiments involving acute R.E. blockade. Untreated blockaded rabbits were again markedly more susceptible to infection than were untreated normal rabbits.

(2) From the condition of the drug-treated normal rabbits at 24 hours after infection, by comparison with the untreated groups, the protective action of sulfathiazole was apparent.

(3) Drug-treated blockaded rabbits succumbed to infection in a manner paralleling the response of untreated blockaded rabbits. There were no indications of a response to sulfathiazole chemotherapy.

(4) The protective action exerted by sulfathiazole in normal rabbits bore no constant relationship to the bacterial populations noted in the blood of these rabbits at the termination of the experiment, and did not parallel the concentration of sulfathiazole present in such blood at that time.

(5) It is our impression that optimal therapeutic response was associated with the higher levels of conjugation of sulfathiazole. No uniform relationships between these two phenomena may be postulated at this time.

Throughout these studies, it has been noted that the variations seen in the response of normal rabbits to infection and to sulfonamide chemotherapy are not demonstrable in the response of blockaded rabbits under similar conditions.

We have concluded that these variations represent varying levels of R.E. efficiency in the individual rabbits studied. By R.E. blockade, there is a tendency toward the reduction of this function to a relatively low and uniform level.

In addition to previous statements based on individual experiments, the following conclusions have been drawn:

(1) An actively functioning R.E.S. is vital to the success of sulfonamide chemotherapy under the conditions described in these experiments. A depression of the function of this system is accompanied by a corresponding depression in the efficiency of sulfanilamide derivatives in the treatment of bacterial infection.

(2) An explanation of the manner in which the functions of the cells of the R.E.S. are manifest in sulfonamide therapy is not available at this time. The value of the findings presented in this investigation to future study is discussed.

(3) Any theory or explanation of the mechanism of action of sulfonamide drugs in combatting bacterial infections must ascribe a primary and predominant role to the functions of the cells of the R.E.S.

VITA

The writer was born in East St. Louis, Illinois, September 23, 1909. He received his primary and secondary education in the public schools of Beardstown, Illinois, and was graduated from Beardstown High School in June, 1927.

He began his pharmacy apprenticeship in 1926, and passed the Illinois Board Examination for Assistant Pharmacists in 1930.

He attended Illinois College, Jacksonville, Illinois, during the school term of 1932-1933. In 1934, he entered the University of Illinois College of Pharmacy in Chicago, and was graduated with High Honors in June, 1937, with the degree of Bachelor of Science in Pharmacy.

He was awarded the Hodel-Saltiel-Hodel prize for general scholarship. He was given the Fairchild Scholarship prize following national competitive examination in August, 1937. He passed the Illinois Board Examination for Registered Pharmacists in 1937.

In September, 1937, he joined the faculty of the University of Illinois College of Pharmacy, and entered the Graduate School on the Chicago campus. Majoring in Pharmacology under Dr. Camp and Dr. McGuigan, he received the degree of Master of Science in October, 1938.

At this writing, he is an Assistant in the Department of Materia Medica and Botany of the College of Pharmacy, and in the Department of Pharmacology of the College of Medicine, University of Illinois.

He is a member of the following societies: Rho Chi, Phi Kappa Phi, and Sigma Xi (active). He is president of the Alumni Association of the College of Pharmacy, and is a member of the American Pharmaceutical Association.

His publications are as follows:

1. (With V. Lindblade and E. H. Wirth) "A Comparative Study of Liquor Carmini and Liquor Cocci," *N. F. Bull.,* 8:10. 1939.
2. (With V. Lindblade and E. H. Wirth) "The Cathartic Action of Aloe," *Ibid.,* 8:54. 1939.
3. (With W. J. R. Camp) "Determination of Sulfanilamide in Tissue, Urine and Blood," *J. Lab. and Clin. Med.,* 24:1198. 1939.
4. "The Chemistry of the Sulfonamides." *Ill. Med. J.,* 78:397. 1940.
5. "The Relation of Hypnotic Drugs to the Toxicity of Sulfanilamide and Sulfapyridine," *Current Researches in Anesthesia and Analgesia* (in press).